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International application number: PCT/IB2006/051199

International filing date: 18 April 2006 (18.04.2006)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/672,900
Filing date: 18 April 2005 (18.04.2005)

Date of receipt at the International Bureau: 12 May 2006 (12.05.2006)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

IB/06/51199
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PA 7008618

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APPLICATION NUMBER: *60/672,900*
FILING DATE: *April 18, 2005*

REC'D 12 MAY 2006
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No. EV 600680256 US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Filippo	Giancotti	New York, NY

Additional inventors are being named on the ______ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Inhibition of tumorigenesis by inhibition of a6b4 integrin

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

[X] The address corresponding to Customer Number: 021121

OR

[ ] Firm or Individual Name: Marina T. Larson, Ph.D

Address		
City	State	Zip
Country	Telephone	Fax

ENCLOSED APPLICATION PARTS *(check all that apply)*

[ ] Application Data Sheet. See 37 CFR 1.76
[ ] CD(s), Number of CDs ______
[X] Specification *Number of Pages* 17
[ ] Other (specify) ______
[X] Drawing(s) *Number of Sheets* 10

Application Size Fee: If the specification and drawings exceed 100 sheets of paper, the application size fee due is $ 250 ($ 125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

METHOD OF PAYMENT OF FILING FEES AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X] Applicant claims small entity status. See 37 CFR 1.27.

TOTAL FEE AMOUNT $ 100.00

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[ ] No.

[X] Yes, the name of the U.S. Government agency and the Government contract number are: NIH Merit Award R13 CA58976

SIGNATURE Marina T. Larson

Date April 18, 2005

TYPED or PRINTED NAME Marina T. Larson, Ph.D

REGISTRATION NO. 32038 *(if appropriate)*

TELEPHONE 970-468-6600

Docket Number: MSK.P-082-PV

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

Inhibition of tumorigenesis by inhibition of a6b4 integrin

Background of Invention

This application relates to a method of inhibiting tumorigenesis, particularly in the case of tyrosine kinase-related cancers such as breast and prostate cancer, through the inhibition of a6b4 integrin using a therapeutic agant that targets the beta 4 portion of the integrin. In this application, the nomenclature a6b4 refers to the alpha-6-beta-4 integrin. Similar nomenclature with arabic or roman numerals is used for other integrins.

Integrins are a class of cellular transmembrane receptors known to bind to extracellular matrix proteins, and therefore they mediate cell-cell and cell-extracellular matrix interactions, referred generally to as cell adhesion events. The integrins connect the extracellular matrix to the intracellular cytoskeleton and cooperate with Receptor Protein Tyrosine Kinases (RPTKs) to regulate cell fate (Giancotti and Ruoslahti, 1999; Hynes, 2003; Miranti and Brugge, 2002). Depending on the integrins they express and the matrix they attach to, normal cells proliferate or undergo growth arrest, migrate or remain stationary, and live or undergo apoptotic death. These effects imply that the integrins impart a stringent control to the action of RPTKs, determining the nature and direction of the cell's response to growth factors and cytokines (Giancotti and Tarone, 2003). Despite considerable amounts of cell biological data, genetic evidence of the significance of integrin signaling remains scarce. In particular, it has been difficult to separate the adhesive and signaling function of individual integrins in any model system analyzed to date.

The integrin receptors constitute a family of proteins with shared structural characteristics of noncovalent heterodimeric glycoprotein complexes formed of alpha and beta subunits. There are eight known beta subunits and fourteen known alpha subunits, which associate in various combinations to form twenty five receptors with different ligand specificities. The ligands for several of the integrins are adhesive extracellular matrix (ECM) proteins such as fibronectin, vitronectin, collagens and laminin. It has been reported that the aVb1 integrin, a fibronectin receptor, and the aV integrins aVb3 and aVb5, which bind to several RGD-cotnaining matrix proteins, promote aniogenesis. Hynes et al. (2002). This property has been considered as a basis for using inhibitors of such integrins as inhibitors of angiogenesis. See US Patents Nos. 5,981,478; 5,766,591; 6,358,970; and 6,645,991. However, while genetic experiments in mice have confirmed the role of a5b1 integrin in angiogenesis, they have not confirmed a role for the aV integrins, thus calling into question the efficacy of anti-angiogeneic therapy based on the latter group. Anti-angiogenic therapy based on

inhibition of a5b1 integrin is problematic because of toxicity arising as a result of the critical involvement of this integrin adhesion of several cell types.

The a6b4 integrin is a laminin-5 receptor expressed by epithelial cells, Schwann cells, and endothelial cells and has several distinguishing features. The cytoplasmic domain of b4 is unusually long (ca. 1000 amino acids) and displays no homology to the short cytoplasmic tails of other b subunits. Upon a6b4 binding to matrix, the unique cytoplasmic domain of b4 is phosphorylated on multiple tyrosines by a Src Family kinase (SFK) and interacts directly with the signaling adaptor protein Shc, causing activation of the Ras to ERK cascade (Dans et al., 2001; Gagnoux-Palacios et al., 2003; Mainiero et al., 1995). In addition, the b4 tail mediates activation of PI-3K and Rac (Shaw, 2001; Shaw et al., 1997). Upon dephosphorylation, the cytoplasmic domain of b4 associates with the keratin cytoskeleton, causing assembly of hemidesmosomes and, hence, strengthening adhesion to laminin-5-containing basement membranes (Dans et al., 2001; Murgia et al., 1998; Spinardi et al., 1993). In contrast, the other integrins activate FAK/SFK signaling at focal adhesions (Geiger et al., 2001; Schlaepfer and Hunter, 1998) and, although some of them also recruit Shc, they do so by a distinct, indirect mechanism (Wary et al., 1998).

The pattern of expression of a6b4 in the skin is consistent with a role for a6b4 signaling in the control of epithelial proliferation. In normal epidermis, the expression of a6b4 is restricted to the basal cell layer, which comprises the rapidly dividing transit-amplifying cells (Borradori and Sonnenberg, 1999; Fuchs et al., 1997), while in skin diseases characterized by suprabasal proliferation, such as squamous carcinoma and psoriasis, a6b4 extends to the suprabasal layers (Pellegrini et al., 1992). In addition, ligation of a6b4 promotes progression through G1 and entry in S phase in keratinocytes treated with EGF, whereas ligation of a2b1 does not exert this effect (Mainiero et al., 1997).

Tumor biology studies have suggested a function for a6b4 signaling in tumor invasion. Many invasive carcinomas display elevated levels of a6b4 (Mercurio and Rabinovitz, 2001). Introduction of a6b4 in breast and colon carcinoma cells that have lost its expression activates PI-3K to Rac signaling and increases invasive ability in vitro (Shaw et al., 1997). In addition, it has been proposed that the b4 tail functions as an essential adapter and amplifier of pro-invasive signals elicited by activated Met in cells undergoing Met-induced oncogenesis (Trusolino et al., 2001). Finally, introduction of a dominant negative form of b4 impairs the survival of breast carcinoma cells, and this effect has been linked to the ability of mutant b4 to interfere with the assembly of hemidesmosomes and the establishment of a partially polarized phenotype (Weaver et al., 2002). Collectively, these results suggest the possibility that a6b4 promotes cell migration and invasion and confers resistance to apoptosis in carcinoma cells.

Commonly assigned US Provisional Application No. 60/481,696, filed November 22, 2003, and PCT application PCT/US2004/039189, which are incorporated herein by reference, described the use of a6b4 inegrin in controlling pathological neogenesis. While this angiogenesis can occur in tumors, controlling angiogenesis is not the same as controlling or inhibiting tumorigenesis and tumor progression.

It has been shown previously that coexpression of a6b4 and laminin and amplficiation of ErbB-2 correlate with a poor prognosis in breast cancer patients (Slamon et al., 1987; Tagliabue et al., 1998). Although a significant fraction of human breast cancers show reduced expression of a6b4, a significant fraction of metastatic lymph nodes stain positive for a6b4 (Natali et al., 1992). Further, there is evidence that introduction of a6b4 increases the invasive ability of MDA-MB-435 breast carcinoma cells in in vitro assays (Shaw et al, 1997) and that the ability of b4-transfected MDA-MB-435 cells to metastasize to lung upon injection into a mouse tail vein requires the binding of a6b4 on cancer cells to Lu-ECAM-1 on lung endothelial cells. (Abdel-DGhany et al., 2001). Pathological studies have shown that the expression of a6b4 declines during prostate cancer progression (Cress et al., Cancer Metastasis Rev. 14:219-228, 1995). However, the integrin is still expressed at significant levels in Prostate Intraepithelial Neoplasia (PIN), and it may play a role at this stage of tumor progression. Nothwithstanding these findings, the role, if any, of a6b4 in tumor progression is not understood in the art. For example, whether the observed variations in expression levels are cause or effect, whether reduction changes I na6b4 have any actual impact of tumor growth or invasiveness in vivo, and how a6b4 interacts with other signaling moieties are not known.

Summary of Invention

Following an investigation of the role of a6b4 in mice engineered to develop mammary tumors on expression of an activated version of ErbB-2 and on mice engineered to develop prostate cancer on expression of the SV-40 T Antigen, we have determined that a6b4 integrin signaling is necessary for the progression of breast and prostate cancer. This finding is also applicable to other tumor types that express a6b4, such as thyroid cancer, squamous carcinoma of the skin, cervix, and upper gastrointestinal tract, pancreatic cancer, colon cancer (Mercurio AM, Rabinovitz I. Towards a mechanistic understanding of tumor invasion--lessons from the alpha6beta 4 integrin. Semin Cancer Biol. 2001 Apr;11(2):129-41). Thus, the present invention provides methods for the inhibition of tumorigenesis in tumors of this type using inhibitors of a6b4 integrin that target beta 4. In accordance with the method of the invention, an individual in whom tumorigensis is to be inhibited is exposed to a therapeutic agent effective to reduce the amount of active a6b4 integrin in the individual, at least at locations relevant to tumorigenesis. In one embodiment of the invention, the individual is a human patient. The therapeutic agent may be an antibody or a small molecule, for example a

laminin-5 analog, which binds to a6b4 integrin and inhibits its normal function. The therapeutic agent may also be a chemical species that interferes with the production of beta 4, including for example an antisense or RNAi species. The therapeutic agent is administered to the tissue or patient in a therapeutically effective amount. The therapeutic agent may be used as a single agent or in combination with other therapies, especially those directed toward suppressing the activity of RPTKs known to cooperate with a6b4, including but not limited to ErbB2, EGF-R, Met, and Ron.

Brief Description of the Drawings

Fig. 1 shows a breeding strategy for introduction of MMTV-Neu^{Ndl}-YD transgene into both wild-type and b4-1355T mice.

Fig. 2 shows the extent of tumor free survival in b4 mutant and wild-type breed in accordance with the scheme in Fig. 1.

Fig. 3 shows the number of individual mammary tumors in individual mice.

Fig. 4 shows the growth of mammary tumors in wild-type and b4-mutant mice.

Fig. 5 shows the difference in histological progression in mammary tumors in wild-type and b4-mutant mice.

Fig. 6 shows a breeding strategy for introduction of TRAMP into both wild-type and b4-1355T mice.

Fig. 7 shows results of an MRI analysis indicative of tumor growth in b4-mutant and b4-wild-type TRAMP mice.

Fig. 8 shows survival of b4-mutant and b4-wild-type TRAMP mice.

Fig. 9 shows the sensitization upon loss of beta=4 siganling when MMTV-Neu (YD) mice bearing mammary tumors were treated with Iressa or vehicle (0.1% Tween-80).

Fig. 10 shows reduction in tumor volume when MMTV-Neu (YD) mice bearing mammary tumors were treated with Iressa.

Detailed Description

As used in this application, the term "tumorigenesis" refers to initiation of primary or metastatic tumor growth, and the promotion of invasive growth.

As used in this application, the term "inhibition" refers to a reduction of the event or activity inhibited to an extent sufficient to produce an observable result. Complete elimination of the event or activity is not required.

As used in this application, the term "amount of active a6b4 integrin" refers to the observable tumorigenesis-promoting activity resulting from a6b4 integrin present in a tissue. Reductions in the amount of the active a6b4 integrin can result from a reduction in the amount of a6b4 integrin, i.e, effectively a reduction in concentration; a reduction in the capacity of individual molecules of a6b4 integrin to promote tumorigenesis, i.e. effectively a change in the quality of the integrin, or combinations thereof. The first type of reduction will most commonly be achieved by limiting the production of a6b4 integrin, for example using a antisense oligonucleotide or RNAi techniques, although it could also be achieved by accelerating the decomposition of a6b4 integrin. The second type of reduction is most readily achieved through physical binding of the integrin with a ligand that competes with the normal ligand for binding to the receptor.

As used in this application, the terms "treatment" or "treating" refer to the application of a therapeutic agent to achieve a reduction in the amount of active a6b4 integrin so as to produce a benefit to a patient being treated. Such a benefit need not be a complete or permanent cure, but may be only a lessening of the rate at which tumorigenesis is occurring, thereby delaying progression of a disease condition.

As used in this application, the term "administration" refers to any means by which a therapeutic agent can be delivered to a tissue, including without limitation oral, nasal and transdermal administration and injection, for example subcutaneous, subdermal, intramuscular, intravenous, intrathecal or peritoneal injection. For treatment of eye-associated tumorgenesis, direct injection to the eye may be used. The therapeutic agent of the invention can be used in combination with other agents used in the treatment of cancer. In particular, the therapeutic agent of the invention is suitably used in combination with kinase inhibitors such as Iressa. Use in combination entails the administration of two or more agents in a time course where the effects of at least one of the agents is improved as a result of the use of the other. Two agents need no be administered at the same time to be considered use in combination, and may be used in any order.

The effective amount of a therapeutic agent to be administered varies depending on the nature of the therapeutic agent, and will frequently reflect a balancing of therapeutic benefits and side effects. However, the determination of specific amounts for a given therapeutic is routine and within the skill in the art.

Therapeutic agents useful in the present invention may be antibodies, aptamers or small molecules that bind to a6b4 integrin to produce a reduction in activity. Examples include small molecules which block b4 signalling by binding to b4, and have specific functions such as inhibiting nuclear translocation of NfkB. Where an antibody therapeutic agent is used, it may be administered in the form of the antibody, or formed in situ by expression of a nucleic acid sequence encoding an a6b4 integrin-specific antibody. Such antibodies may be monoclonal, polyclonal, or modified constructs, for example single chain Fv constructs, targeting a6b4 integrin. Binding sites may be on the alpha chain, the beta chain or both chains of the a6b4 integrin. Non-antibody binding proteins could also be employed. For example, human integrin-beta-4 binding protein is known and has the sequence:

MAVRASFENNCEIGCFAKLTNTYCLVAIGGSENFYSVFEGELSDTIPVVHASIAGCRIIGRMCVGNRHG
LLVPNNTTDQELQHIRNSLPDTVQIRRVEERLSALGNVTTCNDYVALVHPDLDRETEEILADVLKVEVF
RQTVADQVLVGSYCVFSNQGGLVHPKTSIEDQDELSSLLQVPLVAGTVNRGSEVIAAGMVVNDWCAF
CGLDTTSTELSVVESVFKLNEAQPSTIATSMRDSLIDSLT(NM_002212). (Seq. ID. No. 1) .

The therapeutic agent may also be a nucleic acid that results in a reduction in the amount of active a6b4 integrin, for example an antisense oligonucleotide or an RNA molecule that works by an RNAi mechanism. The nucleic acid may target, via a sequence specific mechanism, the alpha chain or the beta chain. The coding sequence of the beta 4 chain of human integrin is known from NM_000213 to be as follows:

1 atggcagggc cacgccccag cccatgggcc aggctgctcc tggcagcctt gatcagcgtc
61 agcctctctg ggaccttggc aaaccgctgc aagaaggccc cagtgaagag ctgcacggag
121 tgtgtccgtg tggataagga ctgcgcctac tgcacagacg agatgttcag ggaccggcgc
181 tgcaacaccc aggcggagct gctggccgcg ggctgccagc gggagagcat cgtggtcatg
241 gagagcagct tccaaatcac agaggagacc cagattgaca ccaccctgcg gcgcagccag
301 atgtcccccc aaggcctgcg ggtccgtctg cggcccggtg aggagcggca ttttgagctg
361 gaggtgtttg agccactgga gagccccgtg gacctgtaca tcctcatgga cttctccaac
421 tccatgtccg atgatctgga caacctcaag aagatggggc agaacctggc tcgggtcctg
481 agccagctca ccagcgacta cactattgga tttggcaagt ttgtggacaa agtcagcgtc
541 ccgcagacgg acatgaggcc tgagaagctg aaggagccct ggcccaacag tgaccccccc
601 ttctccttca agaacgtcat cagcctgaca gaagatgtgg atgagttccg gaataaactg
661 cagggagagc ggatctcagg caacctggat gctcctgagg gcggcttcga tgccatcctg
721 cagacagctg tgtgcacgag ggacattggc tggcgcccgg acagcaccca cctgctggtc
781 ttctccaccg agtcagcctt ccactatgag gctgatggcg ccaacgtgct ggctggcatc
841 atgagccgca acgatgaacg gtgccacctg gacaccacgg gcacctacac ccagtacagg
901 acacaggact acccgtcggt gcccaccctg gtgcgcctgc tcgccaagca caacatcatc
961 cccatctttg ctgtcaccaa ctactcctat agctactacg agaagcttca cacctatttc
1021 cctgtctcct cactgggggt gctgcaggag gactcgtcca acatcgtgga gctgctggag

1081 gaggccttca atcggatccg ctccaacctg gacatccggg ccctagacag cccccgaggc
1141 cttcggacag aggtcacctc caagatgttc cagaagacga ggactgggtc ctttcacatc
1201 cggcgggggg aagtgggtat ataccaggtg cagctgcggg cccttgagca cgtggatggg
1261 acgcacgtgt gccagctgcc ggaggaccag aagggcaaca tccatctgaa accttccttc
1321 tccgacggcc tcaagatgga cgcgggcatc atctgtgatg tgtgcacctg cgagctgcaa
1381 aaagaggtgc ggtcagctcg ctgcagcttc aacggagact tcgtgtgcgg acagtgtgtg
1441 tgcagcgagg gctggagtgg ccagacctgc aactgctcca ccggctctct gagtgacatt
1501 cagccctgcc tgcgggaggg cgaggacaag ccgtgctccg gccgtgggga gtgccagtgc
1561 gggcactgtg tgtgctacgg cgaaggccgc tacgagggtc agttctgcga gtatgacaac
1621 ttccagtgtc cccgcacttc cgggttcctg tgcaatgacc gaggacgctg ctccatgggc
1681 cagtgtgtgt gtgagcctgg ttggacaggc ccaagctgtg actgtcccct cagcaatgcc
1741 acctgcatcg acagcaatgg gggcatctgt aatggacgtg gccactgtga gtgtggccgc
1801 tgccactgcc accagcagtc gctctacacg gacaccatct gcgagatcaa ctactcggcg
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1921 ggcgagaaga aggggcgcac gtgtgaggaa tgcaacttca aggtcaagat ggtggacgag
1981 cttaagagag ccgaggaggt ggtggtgcgc tgctccttcc gggacgagga tgacgactgc
2041 acctacagct acaccatgga aggtgacggc gcccctgggc ccaacagcac tgtcctggtg
2101 cacaagaaga aggactgccc tccgggctcc ttctggtggc tcatcccccct gctcctcctc
2161 ctcctgccgc tcctggccct gctactgctg ctatgctgga agtactgtgc ctgctgcaag
2221 gcctgcctgg cacttctccc gtgctgcaac cgaggtcaca tggtgggctt taaggaagac
2281 cactacatgc tgcgggagaa cctgatggcc tctgaccact tggacacgcc catgctgcgc
2341 agcgggaacc tcaagggccg tgacgtggtc cgctggaagg tcaccaacaa catgcagcgg
2401 cctggctttg ccactcatgc cgccagcatc aaccccacag agctggtgcc ctacgggctg
2461 tccttgcgcc tggcccgcct ttgcaccgag aacctgctga agcctgacac tcgggagtgc
2521 gcccagctgc gccaggaggt ggaggagaac ctgaacgagg tctacaggca gatctccggt
2581 gtacacaagc tccagcagac caagttccgg cagcagccca atgccgggaa aaagcaagac
2641 cacaccattg tggacacagt gctgatggcg ccccgctcgg ccaagccggc cctgctgaag
2701 cttacagaga agcaggtgga acagagggcc ttccacgacc tcaaggtggc cccccggctac
2761 tacaccctca ctgcagacca ggacgcccgg ggcatggtgg agttccagga gggcgtggag
2821 ctggtggacg tacgggtgcc cctctttatc cggcctgagg atgacgacga gaagcagctg
2881 ctggtggagg ccatcgacgt gcccgcaggc actgccaccc tcggccgccg cctggtaaac
2941 atcaccatca tcaaggagca agccagagac gtggtgtcct ttgagcagcc tgagttctcg
3001 gtcagccgcg gggaccaggt ggcccgcatc cctgtcatcc ggcgtgtcct ggacggcggg
3061 aagtcccagg tctcctaccg cacacaggat ggcaccgcgc agggcaaccg ggactacatc
3121 cccgtggagg gtgagctgct gttccagcct ggggaggcct ggaaagagct gcaggtgaag
3181 ctcctggagc tgcaagaagt tgactccctc ctgcggggcc gccaggtccg ccgtttccac
3241 gtccagctca gcaaccctaa gtttggggcc cacctgggcc agccccactc caccaccatc
3301 atcatcaggg acccagatga actggaccgg agcttcacga gtcagatgtt gtcatcacag
3361 ccacccccctc acggcgacct gggcgccccg cagaacccca atgctaaggc cgctgggtcc
3421 aggaagatcc atttcaactg gctgcccccct tctggcaagc caatggggta cagggtaaag
3481 tactggattc agggcgactc cgaatccgaa gcccacctgc tcgacagcaa ggtgccctca
3541 gtggagctca ccaacctgta cccgtattgc gactatgaga tgaaggtgtg cgcctacggg

3601 gctcagggcg agggacccta cagctccctg gtgtcctgcc gcacccacca ggaagtgccc
3661 agcgagccag ggcgtctggc cttcaatgtc gtctcctcca cggtgaccca gctgagctgg
3721 gctgagccgg ctgagaccaa cggtgagatc acagcctacg aggtctgcta tggcctggtc
3781 aacgatgaca accgacctat tgggcccatg aagaaagtgc tggttgacaa ccctaagaac
3841 cggatgctgc ttattgagaa ccttcgggag tcccagccct accgctacac ggtgaaggcg
3901 cgcaacgggg ccggctgggg gcctgagcgg gaggccatca tcaacctggc cacccagccc
3961 aagaggccca tgtccatccc catcatccct gacatcccta tcgtggacgc ccagagcggg
4021 gaggactacg acagcttcct tatgtacagc gatgacgttc tacgctctcc atcgggcagc
4081 cagaggccca gcgtctccga tgacactggc tgcggctgga agttcgagcc cctgctgggg
4141 gaggagctgg acctgcggcg cgtcacgtgg cggctgcccc cggagctcat cccgcgcctg
4201 tcggccagca gcgggcgctc ctccgacgcc gaggccccca cggccccccg gacgacggcg
4261 gcgcgggcgg gaagggcggc agccgtgccc cgcagtgcga cacccgggcc ccccggagag
4321 cacctggtga atggccggat ggactttgcc ttcccgggca gcaccaactc cctgcacagg
4381 atgaccacga ccagtgctgc tgcctatggc acccacctga gcccacacgt gccccaccgc
4441 gtgctaagca catcctccac cctcacacgg gactacaact cactgacccg ctcagaacac
4501 tcacactcga ccacactgcc cagggactac tccaccctca cctccgtctc ctccccacgac
4561 tctcgcctga ctgctggtgt gcccgacacg cccacccgcc tggtgttctc tgccctgggg
4621 cccacatctc tcagagtgag ctggcaggag ccgcggtgcg agcggccgct gcagggctac
4681 agtgtggagt accagctgct gaacggcggt gagctgcatc ggctcaacat ccccaaccct
4741 gcccagacct cggtggtggt ggaagacctc ctgcccaacc actcctacgt gttccgcgtg
4801 cgggcccaga gccaggaagg ctggggccga gagcgtgagg gtgtcatcac cattgaatcc
4861 caggtgcacc cgcagagccc actgtgtccc ctgccaggct ccgccttcac tttgagcact
4921 cccagtgccc caggcccgct ggtgttcact gccctgagcc cagactcgct gcagctgagc
4981 tgggagcggc cacggaggcc caatggggat atcgtcggct acctggtgac ctgtgagatg
5041 gcccaaggag gagggccagc caccgcattc cgggtggatg gagacagccc cgagagccgg
5101 ctgaccgtgc cgggcctcag cgagaacgtg ccctacaagt tcaaggtgca ggccaggacc
5161 actgagggct tcgggccaga gcgcgagggc atcatcacca tagagtccca ggatggagga
5221 cccttcccgc agctgggcag ccgtgccggg ctcttccagc acccgctgca aagcgagtac
5281 agcagcatca ccaccaccca caccagcgcc accgagccct tcctagtgga tgggctgacc
5341 ctgggggccc agcacctgga ggcaggcggc tccctcaccc ggcatgtgac ccaggagttt
5401 gtgagccgga cactgaccac cagcggaacc cttagcaccc acatggacca acagttcttc
5461 caaacttga (Seq. ID. No. 2)

The coding sequence of the alpha 6 chain of human integrin is known from NM_000210 to be as follows:

1 atggccgccg ccgggcagct gtgcttgctc tacctgtcgg cggggctcct gtcccggctc
61 ggcgcagcct tcaacttgga cactcgggag gacaacgtga tccggaaata tggagacccc
121 gggagcctct tcggcttctc gctggccatg cactggcaac tgcagcccga ggacaagcgg
181 ctgttgctcg tgggggcccc gcgcggagaa gcgcttccac tgcagagagc caacagaacg
241 ggagggctgt acagctgcga catcaccgcc cgggggccat gcacgcggat cgagtttgat
301 aacgatgctg accccacgtc agaaagcaag gaagatcagt ggatgggggt caccgtccag

361 agccaaggtc cagggggcaa ggtcgtgaca tgtgctcacc gatatgaaaa aaggcagcat
421 gttaatacga agcaggaatc ccgagacatc tttgggcggt gttatgtcct gagtcagaat
481 ctcaggattg aagacgatat ggatggggga gattggagct tttgtgatgg gcgattgaga
541 ggccatgaga aatttggctc ttgccagcaa ggtgtagcag ctacttttac taaagacttt
601 cattacattg tatttggagc cccgggtact tataactgga aagggattgt tcgtgtagag
661 caaaagaata acactttttt tgacatgaac atctttgaag atgggcctta tgaagttggt
721 ggagagactg agcatgatga aagtctcgtt cctgttcctg ctaacagtta cttaggtttt
781 tctttggact cagggaaagg tattgtttct aaagatgaga tcacttttgt atctggtgct
841 cccagagcca atcacagtgg agccgtggtt ttgctgaaga gagacatgaa gtctgcacat
901 ctcctccctg agcacatatt cgatggagaa ggtctggcct cttcatttgg ctatgatgtg
961 gcggtggtgg acctcaacaa ggatgggtgg caagatatag ttattggagc cccacagtat
1021 tttgatagag atggagaagt tggaggtgca gtgtatgtct acatgaacca gcaaggcaga
1081 tggaataatg tgaagccaat tcgtcttaat ggaaccaaag attctatgtt tggcattgca
1141 gtaaaaaata ttggagatat taatcaagat ggctacccag atattgcagt tggagctccg
1201 tatgatgact tgggaaaggt tttatctat catggatctg caaatggaat aaataccaaa
1261 ccaacacagg ttctcaaggg tatatcacct tattttggat attcaattgc tggaaacatg
1321 gaccttgatc gaaattccta ccctgatgtt gctgttggtt ccctctcaga ttcagtaact
1381 attttcagat cccggcctgt gattaatatt cagaaaacca tcacagtaac tcctaacaga
1441 attgacctcc gccagaaaac agcgtgtggg gcgcctagtg ggatatgcct ccaggttaaa
1501 tcctgttttg aatatactgc taaccccgct ggttataatc cttcaatatc aattgtgggc
1561 acacttgaag ctgaaaaaga aagaagaaaa tctgggctat cctcaagagt tcagtttcga
1621 aaccaaggtt ctgagcccaa atatactcaa gaactaactc tgaagaggca gaaacagaaa
1681 gtgtgcatgg aggaaaccct gtggctacag gataatatca gagataaact gcgtcccatt
1741 cccataactg cctcagtgga gatccaagag ccaagctctc gtaggcgagt gaattcactt
1801 ccagaagttc ttccaattct gaattcagat gaacccaaga cagctcatat tgatgttcac
1861 ttcttaaaag agggatgtgg agacgacaat gtatgtaaca gcaaccttaa actagaatat
1921 aaattttgca cccgagaagg aaatcaagac aaattttctt atttaccaat tcaaaaaggt
1981 gtaccagaac tagttctaaa agatcagaag gatattgctt tagaaataac agtgacaaac
2041 agcccttcca acccaaggaa tcccacaaaa gatggcgatg acgcccatga ggctaaactg
2101 attgcaacgt ttccagacac tttaacctat tctgcatata gagaactgag ggctttccct
2161 gagaaacagt tgagttgtgt tgccaaccag aatggctcgc aagctgactg tgagctcgga
2221 aatcctttta aaagaaattc aaatgtcact ttttatttgg ttttaagtac aactgaagtc
2281 acctttgaca ccccatatct ggatattaat ctgaagttag aaacaacaag caatcaagat
2341 aatttggctc caattacagc taaagcaaaa gtggttattg aactgctttt atcggtctcg
2401 ggagttgcta aaccttccca ggtgtatttt ggaggtacag ttgttggcga gcaagctatg
2461 aaatctgaag atgaagtggg aagtttaata gagtatgaat tcagggtaat aaacttaggt
2521 aaacctctta caaacctcgg cacagcaacc ttgaacattc agtggccaaa agaaattagc
2581 aatgggaaat ggttgcttta tttggtgaaa gtagaatcca aaggattgga aaaggtaact
2641 tgtgagccac aaaaggagat aaactccctg aacctaacgg agtctcacaa ctcaagaaag
2701 aaacgggaaa ttactgaaaa acagatagat gataacagaa aattttcttt atttgctgaa
2761 agaaaatacc agactcttaa ctgtagcgtg aacgtgaact gtgtgaacat cagatgcccg
2821 ctgcgggggc tggacagcaa ggcgtctctt attttgcgct cgaggttatg gaacagcaca

2881 tttctagagg aatattccaa actgaactac ttggacattc tcatgcgagc cttcattgat
2941 gtgactgctg ctgccgaaaa tatcaggctg ccaaatgcag gcactcaggt tcgagtgact
3001 gtgtttccct caaagactgt agctcagtat tcgggagtac cttggtggat catcctagtg
3061 gctattctcg ctgggatctt gatgcttgct ttattagtgt ttatactatg gaagtgtggt
3121 ttcttcaaga gaaataagaa agatcattat gatgccacat atcacaaggc tgagatccat
3181 gctcagccat ctgataaaga gaggcttact tctgatgcat ag

(Seq. ID. No. 3)

Antisense and RNAi sequence are derivable from these sequences. Antisense oligonucleotides are commonly from 12 to 50 bases in length, more preferably 15-30 bases length. Effective regions for targeting of antisense sequences may be found throughout the target nucleic acid. A preferred intragenic site is the region encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene. Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used in vivo to initiate translation of an mRNA molecule transcribed from a gene encoding Integrin beta 4, regardless of the sequence(s) of such codons.

It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively). The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon.

The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination

codon, is also a region which may be targeted effectively. Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on the gene, and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap. The 5' cap region may also be a preferred target region.

RNAi molecules are similarly selected based on the sequence and defined parameters known for the selection of appropriate sequences. RNAi molecules may be single or double stranded, and generally have a length of 19 to 23 bases, although longer and shorter species can be used. A specific RNAi species useful in the method of the invention is based on the mouse sequence of beta-4 cDNA (Genebank Acc. # L04678): nucleotides 113 to 131, counting from the A of the ATG translational start site, having the sequence GAGCTGTACCGAGTGCATC (Seq. ID. No. 4). This molecule, and the corresponding molecule based on the human sequence, and their use form a further aspect of this invention.

In one embodiment of the invention, the therapeutic agent is in combination with other therapy directed toward suppressing the activity of RPTKs known to cooperate with a6b4, including but not limited to ErbB2 (Her2), EGF-R, Met, and Ron. Specific examples of such inhibitors include the Her2 inhibitor trastuzumab (Herceptin^{tm}), PPAR gamma ligands as described in US Patent No. 6,291,496, which is incorporated herein by reference

The invention and the evidence that established the efficacy and utility of the invention will now be further described with reference to the following non-limiting examples.

Example 1

The MMTV-Neu^{Ndl}-YD transgene was introduced into both wild-type and b4-1355T mice of FVB background using the breeding strategy outlined in Fig. 1 (asterick points to Neu mutant. Tumor onset was evaluated by palpation, and mice carrying palpable mammary nodules considered affect. As shown in Fig. 2, the b4 mutant mice lived free of tumors significantly longer than the corresponding control mice. In addition, the b4 mutant mice developed, on average, a smaller number of individual tumors in their

mammary glands. (Fig. 3). These results indicate that a6b4 signalling promotes tumorigenesis in this model of breast cancer.

Example 2

Tumor growth was evaluated at 6 to 8 weeks after initial detection of tumors. As shown in Fig. 4, the mmary carcinomas of b4 mutant mice grew at a slower rate than those of the control mice, indicating that a6b4 signalling promotes tumor growth. Histological analysis indicated that tumors arising in the b4 mutants background were significantly more differentiated (mostly adenocarcinomas) that those arising in the the wild-type background (mostly undifferentiated invasive carcinomas). (Fig. 5) Furthermore, immunohistochemistry showed that the b4 mutant retained an apparently intact laminin-containing basement membrane, whereas the wildtype had disrupted the basement membrane and progressed to a frankly invasive stage.

Example 3

To begin to examine the mechanism by which a6b4 signalling promotes tumor progression, we isolated mammary tumor cell lines from both wild-type and b4 mutant mice. Upon plating on a 2-D matrix, control and b4 mutant tumor cells grew at similar rates. However, when suspended in a Matrigel (a 3-D gel containing basement membrane components) the wild-type tumors proliferated rapidly, producing disorganized aggregates. In contrast, the b4 mutant cells gave rise to small cystic structures resembling normal mammary acini. Taken together, these results provide, for the first time, genetic evidence that a4b6 signalling accelerates breast cancer progression by promoting the transition from adenocarcinoma in situ to invasive and metastatic carcinoma and by promoting tumor growth.

Example 4

Transgenic mice expressing an SV-40-Tag oncogene from the prostate-specific promoter of Probasin (TRAMP mice) develop prostate cancer with complete pentrance (Gingrich et al., 1992). To example the role of a6b4 signalling in prostate carcinoma progression, we introduced the Probasin-SV-40-Tag trfansgene in both wild-type and b4-mutant mice following the breeding strategy outlined in Fig. 6. MRI analysis indicated that tumor onset and growth were delaying in mice carrying the b4 mutation as compared to mice expressing mild-type b4. (Fig. 7). In addition, the overall survival of the b4 mutant TRAMP mice was observed to be longer than that of the b4-wild-type TRAMP mice. (Fig. 8).

Example 5

Consistent with the results in mammary tumors, histological analysis of prostate tumors from b4-mutant and b4-wild-type TRAMP mice indicated that the tumors arising in the b4-mutant background were considerably more differentiated than those arising in the b4- wild-type background. Anti-Ki67 staining, which marks the nuclei of proliferating cells, revealed that b4-mutant tumors have a significantly reduced prolliferative index as compared to the b4-wild-type tumors. Furthermore, b4 was polarized in correspondence of the basement membrane in the tumors of b4-mutant but not b4-wild-type background. Taken together, these findings demonstrate that in the prostate, as in the mammary gland, a6b4 signalling promotes the transition from well differentiated adenocarcinoma with intact basement membrane to invasive carcinoma.

Example 6

We have treated with the kinase inhibitor IRESSA, which inhibits both the EGF-R and Neu (de Bono and Rowinsky, 2002), wild-type and β4-mutant mice carrying MMTV-Neu tumors. The results indicated that IRESSA causes a much larger inhibition of tumor cell proliferation in β4-mutant mice than it does in wild-type mice (Figure 9). About 80 % of the IRESSA-treated tumors arising in β4-mutant mice regress, compared to about only 20 % in the control group (Figure 2). This striking result indicates that blockage of α6β4 increases the effectiveness of cancer therapy with RTK inhibitors, a group which includes all tumor types expressing α6β4 and carrying amplified or activated versions of Neu, EGF-R, and Met RTKs (Bacus et al., 1994; Longati et al., 2001; Sawyers, 2002).

MMTV-Neu (YD) mice bearing mammary tumors (>0.5 cm in diameter) were treated with Iressa (100 mg/Kg/day) or vehicle (0.1% Tween-80) by gastric gavage for 1 month or 7 days. Tumor sections were stained with anti-Ki-67 Mab, which labels proliferating cells. There is no significant difference in Ki-67 staining between mice treated for 1 month or 7 days. Unpaired, two-tailed t-test showed: $P<0.01$ between WT-vehicle and 1355T-vehicle; $P<0.001$ between WT-Iressa and 1355T-Iressa; $P=0.016$ between 1355T-vehicle and 1355T-Iressa. (Fig. 9)

MMTV-Neu (YD) mice bearing mammary tumors (>0.5cm in diameter) were treated with Iressa (100 mg/Kg/day) by gastric gavage for 24 days. Tumor volumes were measured by caliper. Left panel shows the fold changes in tumor volume from day 0 to day 24, with each line representing one mouse (N=10 for each group, $P<0.01$). Right panel of Fig. 10 shows the percentage of mice with regressed tumors in each group.

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Based on the foregoing, it will be understood that the present invention provides the following advances in the art:

1. A method for inhibition of tumorigenesis in an individual suffering from or at risk for a tumor type that expresses a6b4 integrin, such as thyroid, breast , prostate and cervical cancers, cancer of the upper gastrointestinal tract or squamous carcinoma of the skin, comprising the steps of administering to the individual a therapeutic agent effective to reduce the amount of active a6b4 integrin at least in a portion of the individual where tumorigenesis may occur by targeting the beta 4 portion of the integrin.

2. The method of paragraph 1, wherein the individual is human.

3. The method of paragraph 1 or 2, wherein the therapeutic agent is an antibody.

3. The method of paragraph 1 or 2, wherein the therapeutic agent is an antisense oligonucleotide.

4. The method of paragraph 1 or 2, wherein the therapeutic agent is an RNAi species.

5. The method of any of paragraphs 1 to 4, further comprising the step of administering to the individual an inhibitor of a receptor protein tyrosine kinase such as ErbB2, EGF-R. Met and Ron.

5. Use of an inhibitor of a6b4 integrin that targets beta 4 in the preparation of a pharmaceutical composition for inhibition of tumorigenesis.

6. Use of paragraph 5, wherein wherein the therapeutic agent is an antibody.

7. Use of paragraph 5, wherein the therapeutic agent is an antisense oligonucleotide.

8. Use of paragraph 5, wherein the therapeutic agent is an RNAi species.

9. Use of any of paragraphs 5 to 8, wherein the pharmaceutical composition is suitable for human administration.

Introduction of MMTV-Neu Transgene in β4 1355T and Wild Type Mice*

$\beta 4^{WT} / \beta 4^{1355T}$; MMTV-Neu* X $\beta 4^{WT} / \beta 4^{1355T}$; MMTV-Neu *

⇩

$\beta 4^{1355T} / \beta 4^{1355T}$; MMTV-Neu + $\beta 4^{WT} / \beta 4^{WT}$; MMTV-Neu

Experimental group — Control group

Fig. 1

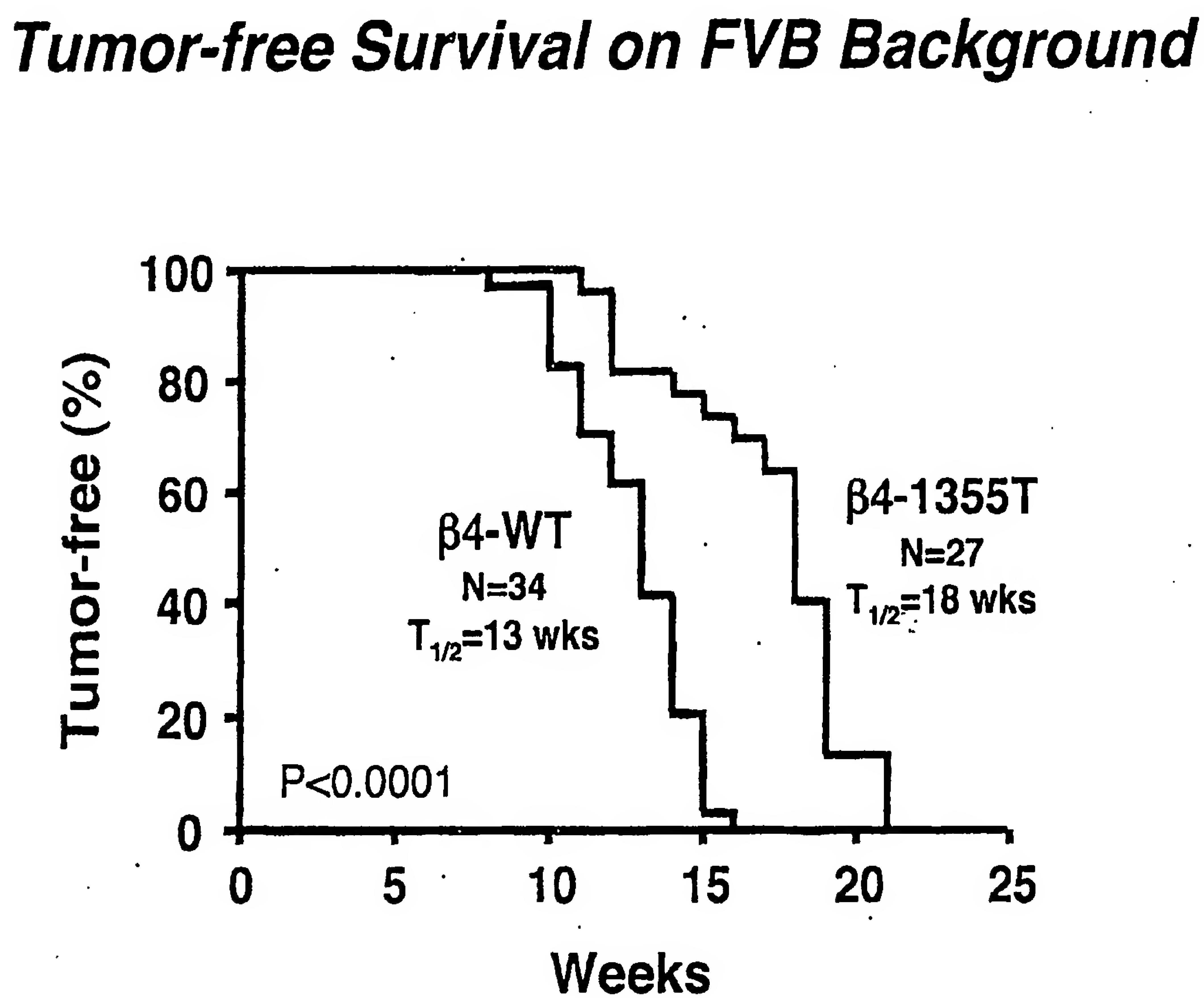

Tumor-free Survival on FVB Background
100
80
60
40
20
0
Tumor-free (%)
β4-WT
N=34
T1/2=13 wks
β4-1355T
N=27
T1/2=18 wks
P<0.0001
0
5
10
15
20
25
Weeks

Fig. 2

The β4-1355T Mice Develop Fewer Mammary Tumors

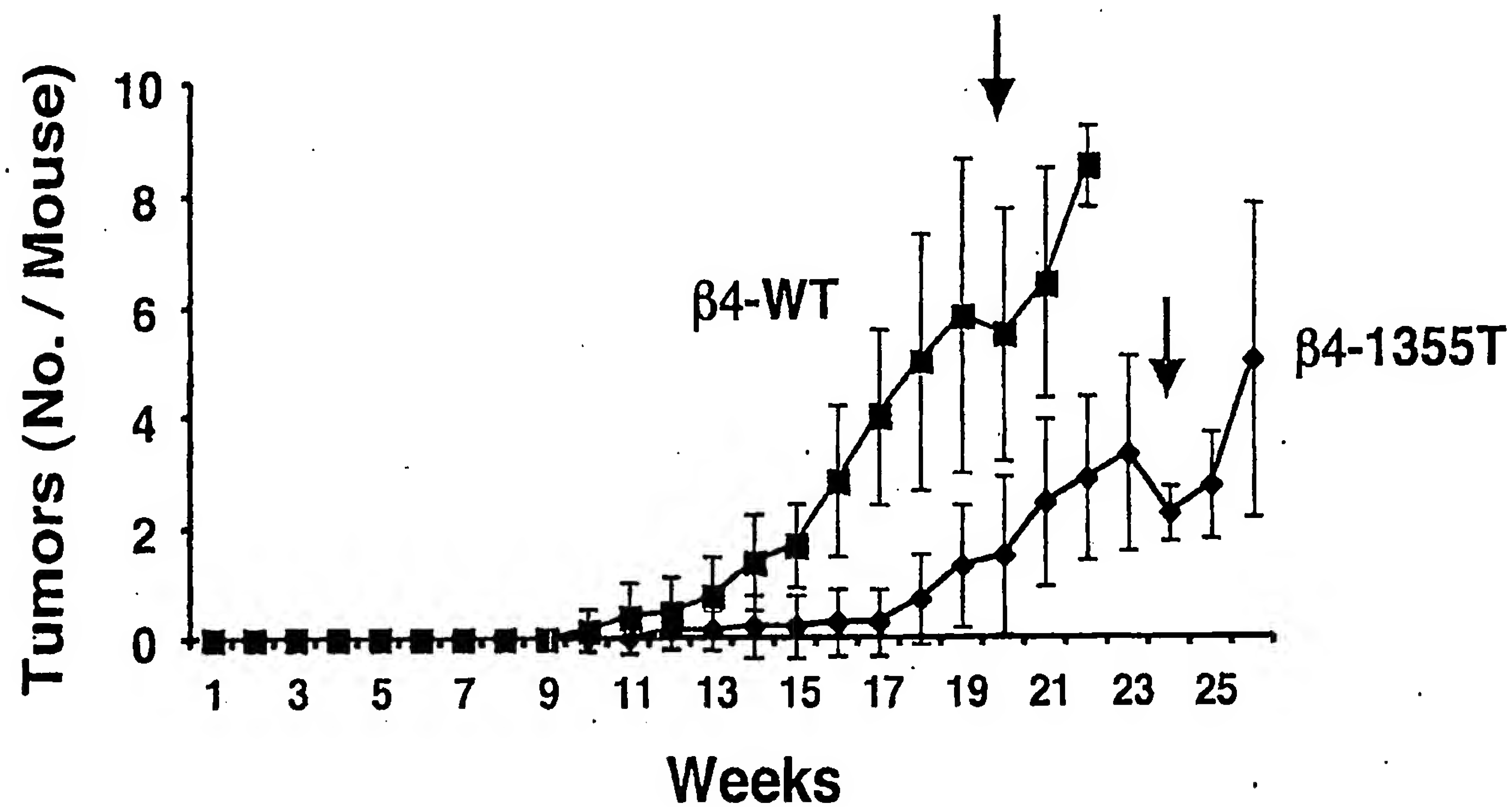
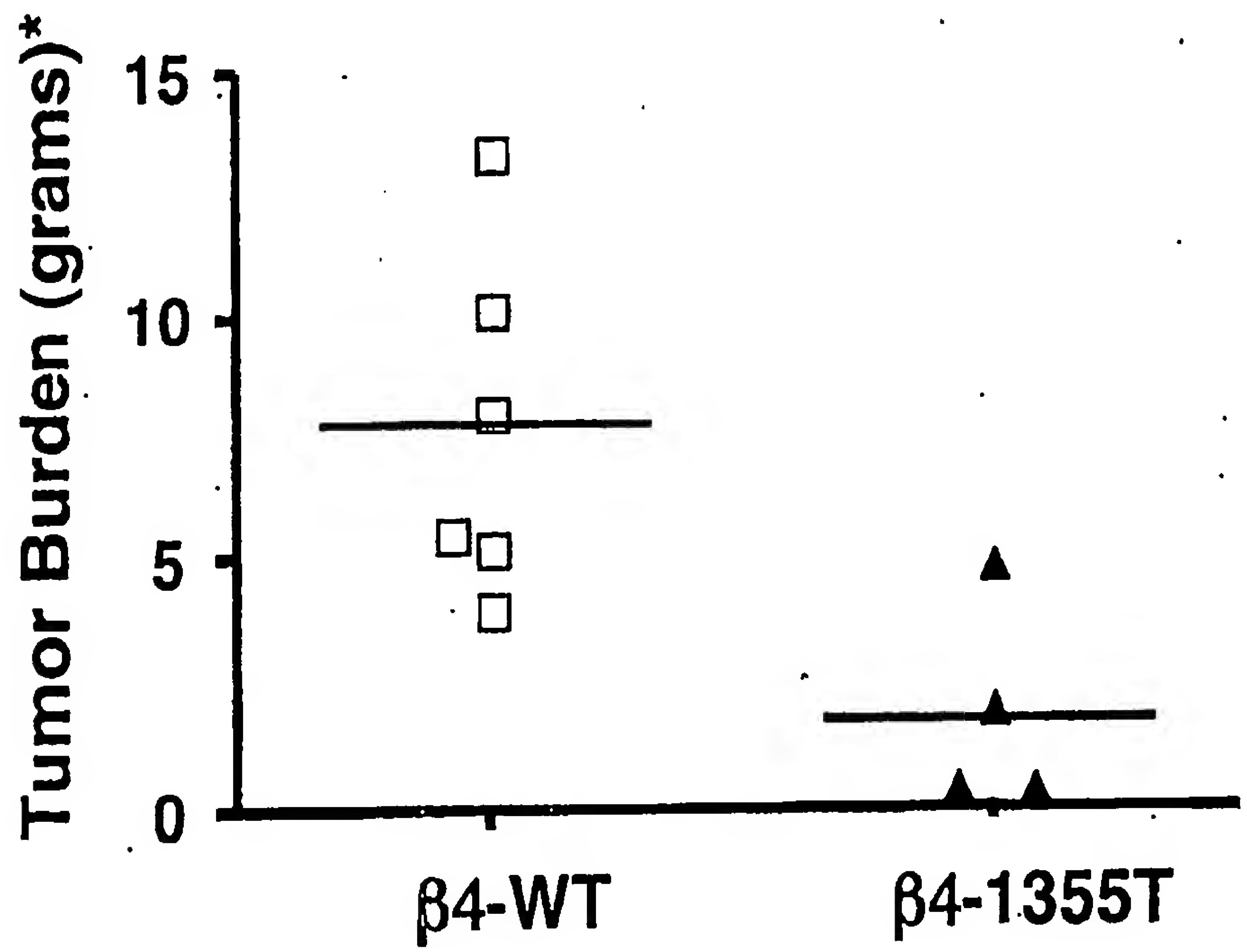


Fig 3

Reduced Mammary Tumor Growth in β4-1355T Mice



* 6-8 weeks after initial palpation

Fig. 4

The Mammary Tumors of β4-1355T Mice are more Differentiated

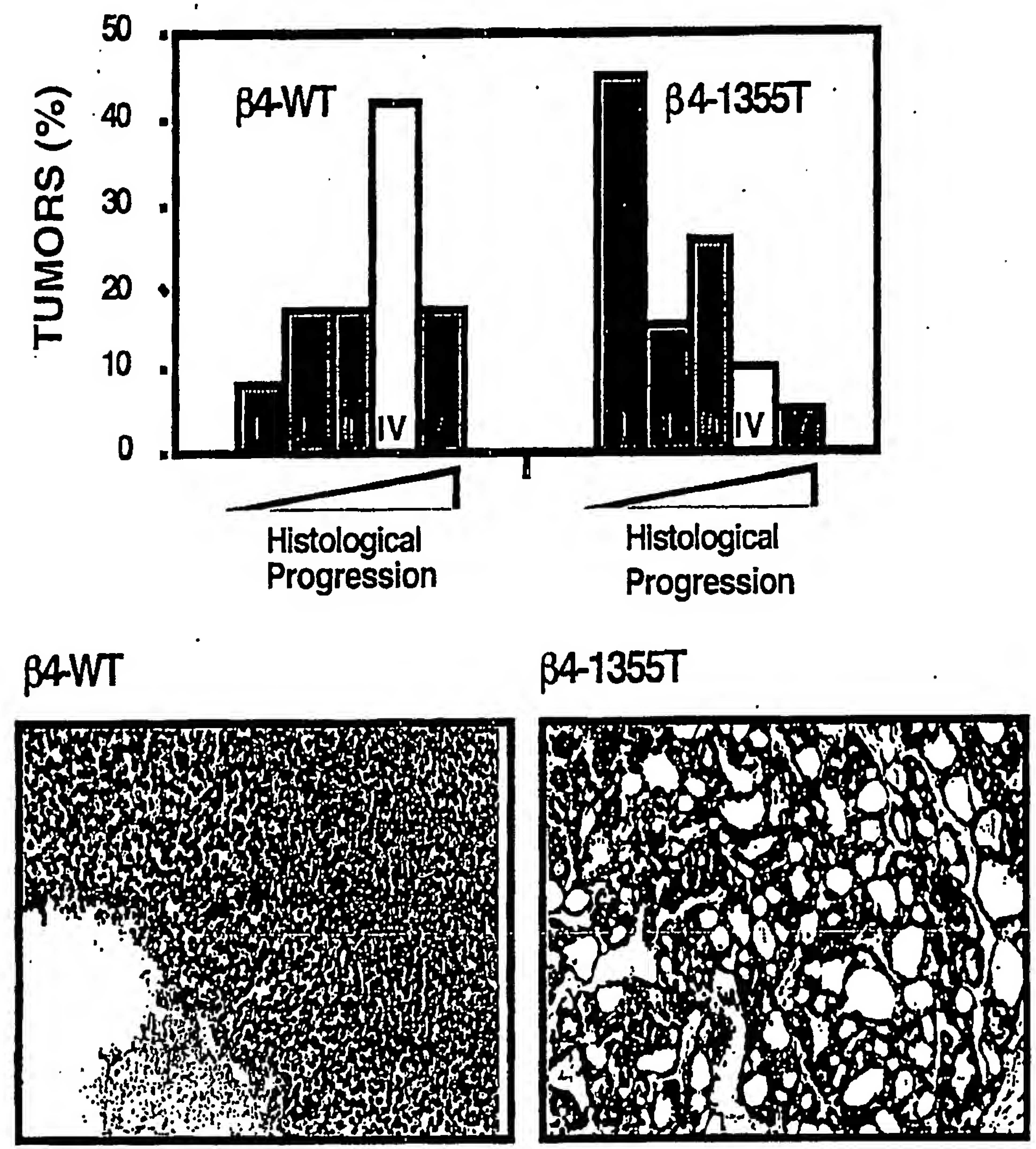


Fig. 5

Introduction of Probasin-SV40-Tag Transgene in $\beta 4$ 1355T and Wild Type Mice

$\beta 4^{WT} / \beta 4^{1355T}$; TRAMP X $\beta 4^{WT} / \beta 4^{1355T}$; TRAMP

$\Downarrow$

$\beta 4^{1355T} / \beta 4^{1355T}$; TRAMP + $\beta 4^{WT} / \beta 4^{WT}$; TRAMP

Experimental group — Control group

Fig. 6

MRI Study of Prostate Ca. Progression in β4-1355T Mice

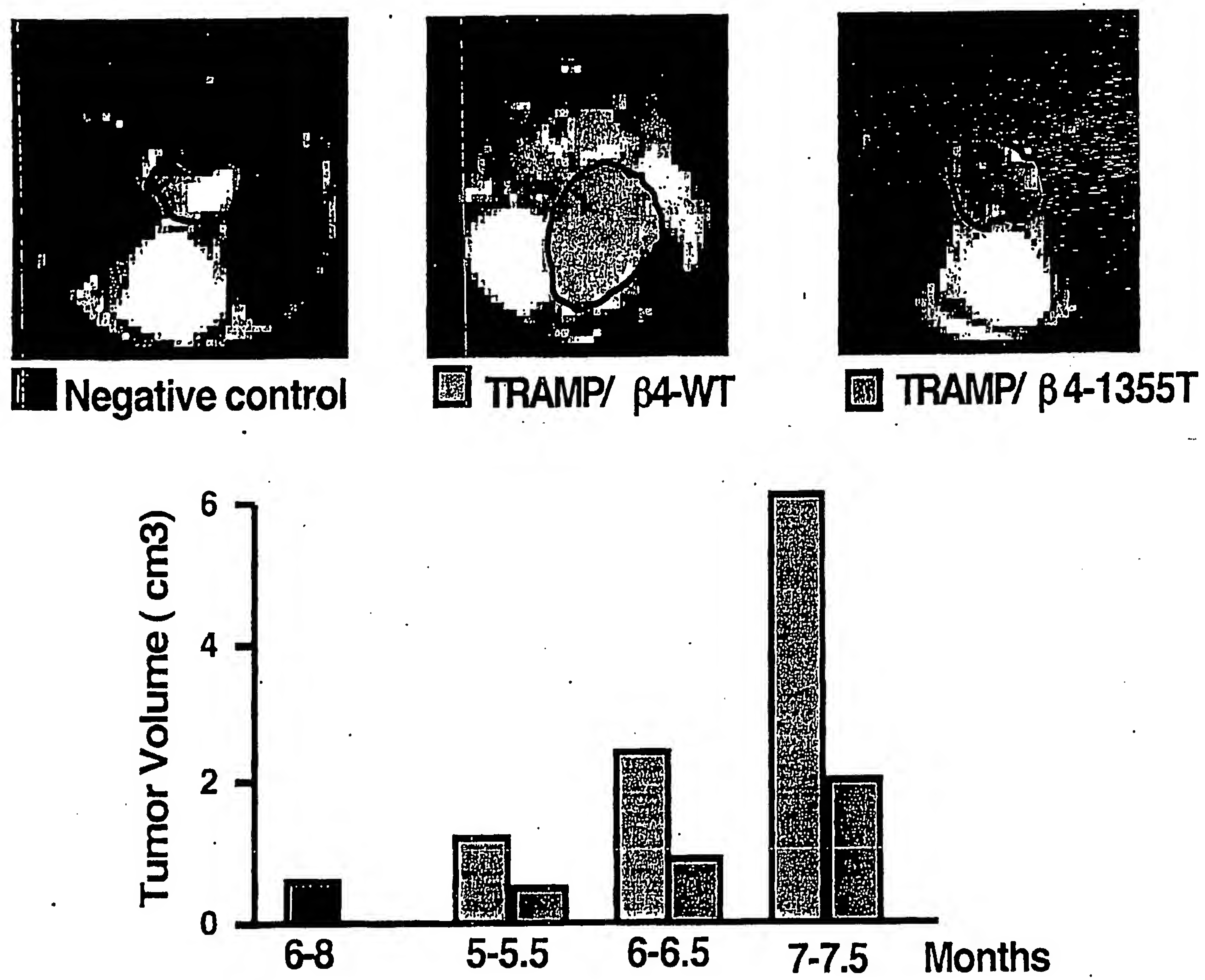


Fig. 7

Delayed Progression of Probasin-SV40-TAg-induced Prostate Carcinomas in β4-1355T Mice

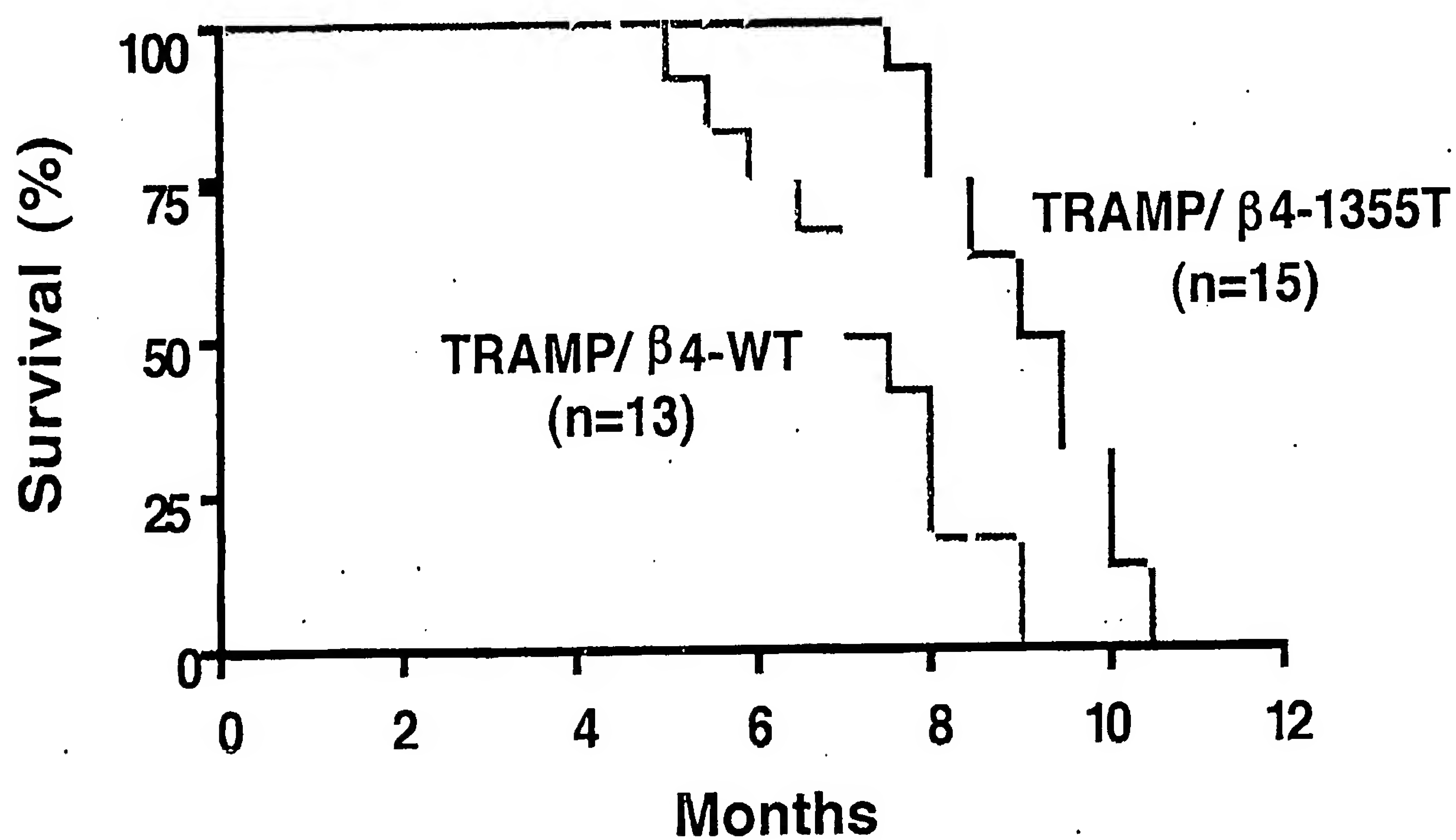


Fig. 8

Loss of β4 signaling sensitizes MMTV-Neu tumors to anti-ErbB targeted therapy

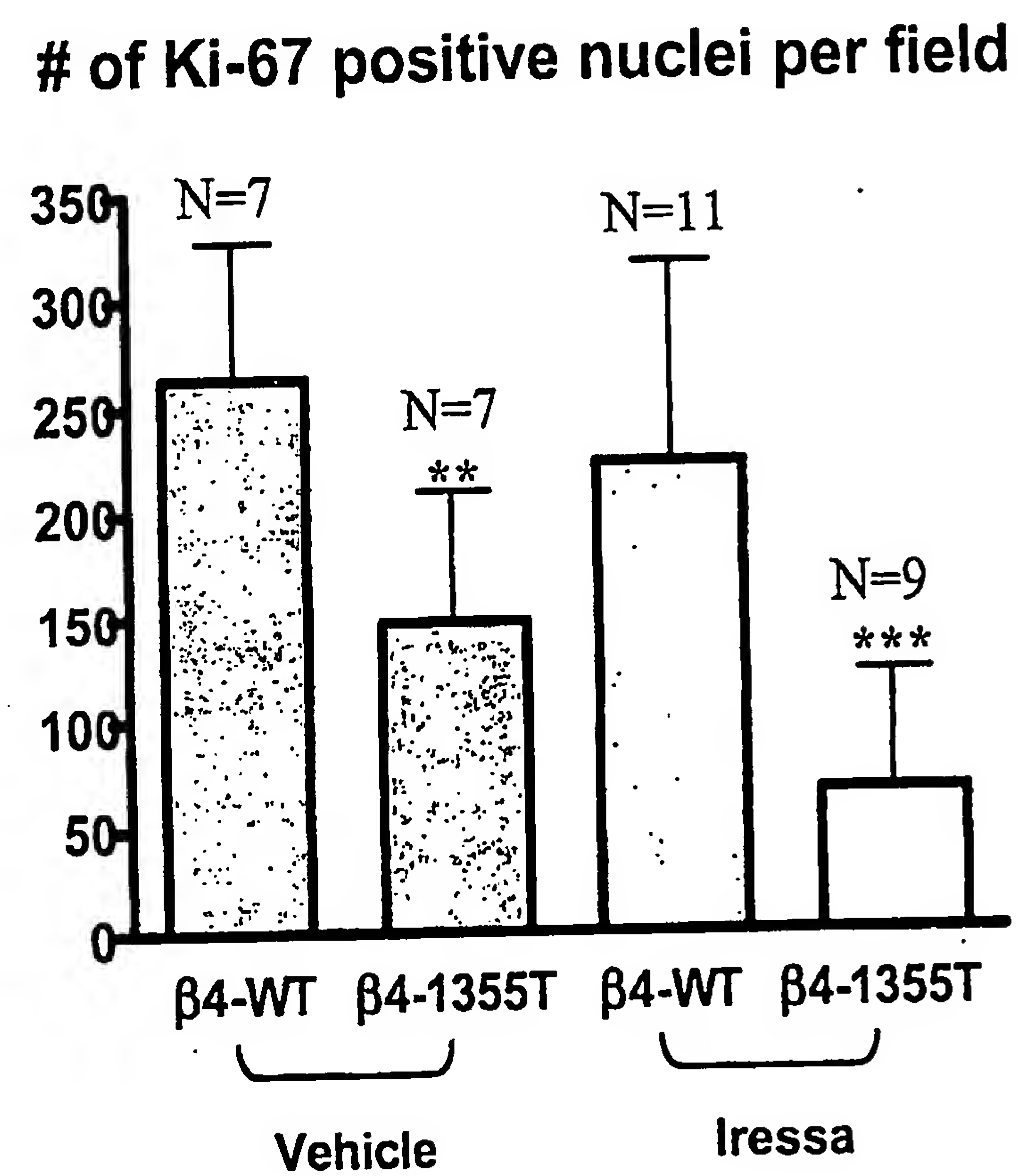
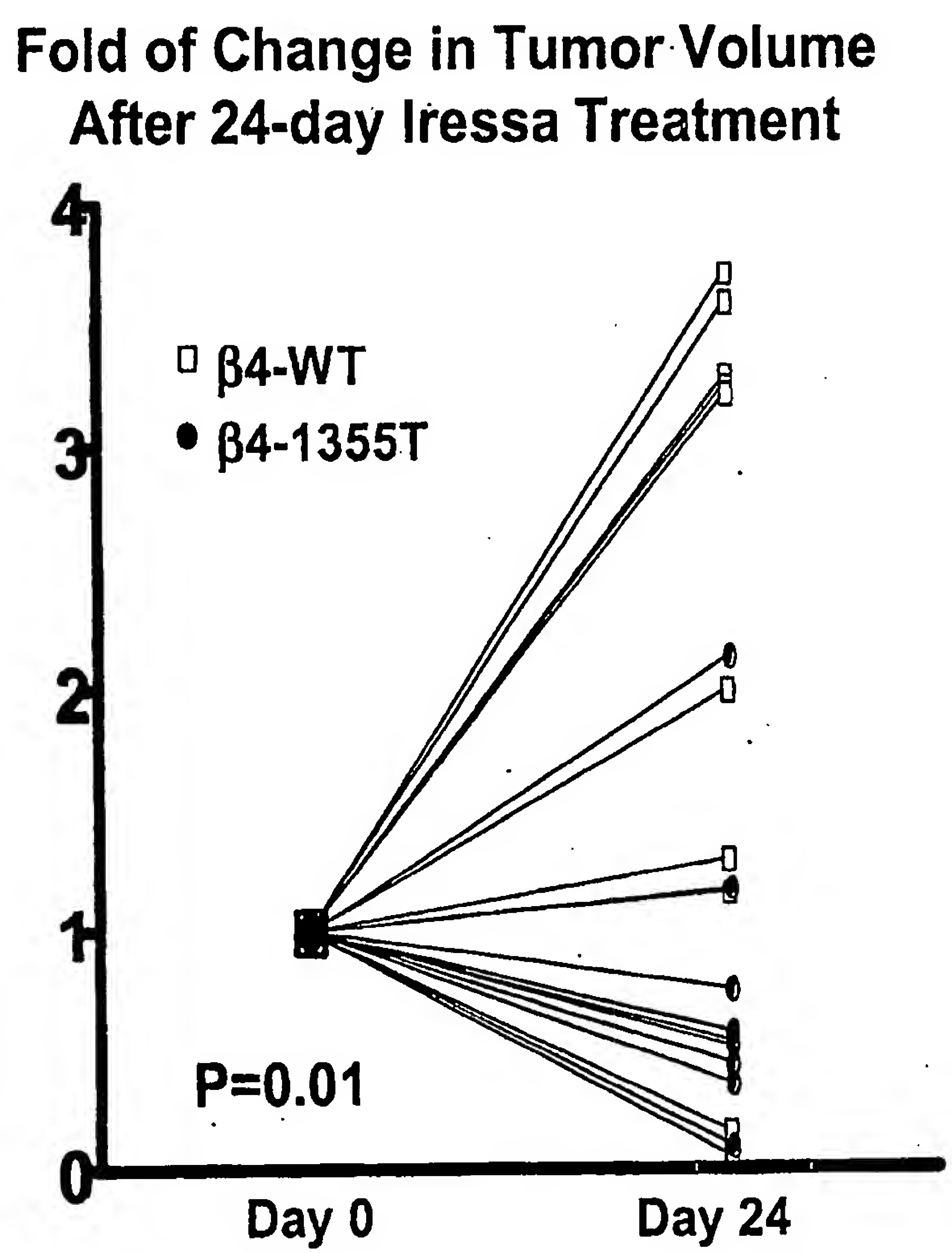
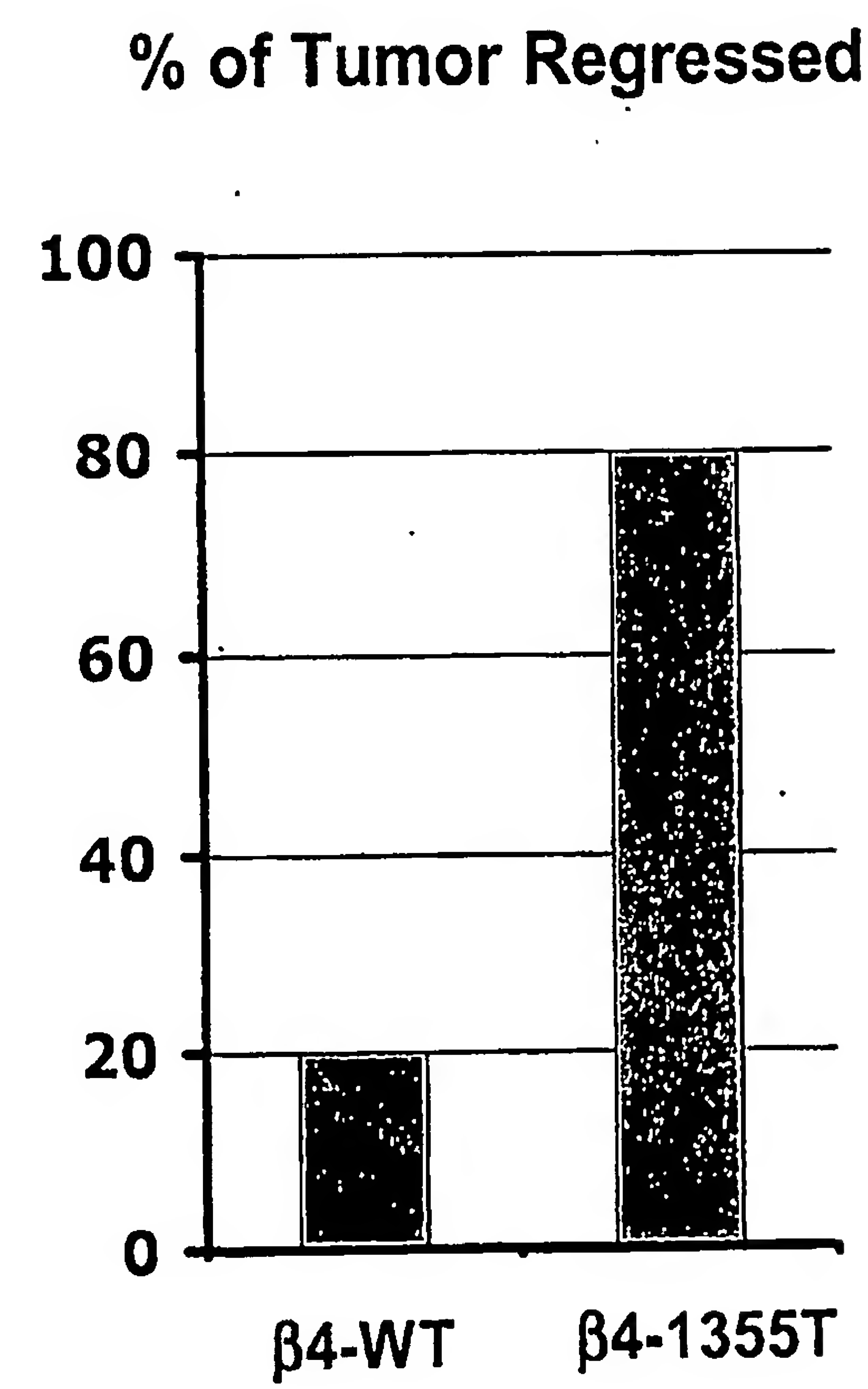


Fig. 9

Fig. 10



Fold of Change in Tumor Volume
After 24-day Iressa Treatment
4
3
2
1
0
β4-WT
β4-1355T
P=0.01
Day 0
Day 24



% of Tumor Regressed
100
80
60
40
20
0
β4-WT
β4-1355T